REMARKS

Upon entry of the above amendment, claims 16-92 will be pending in the application, claims 1-15 have been cancelled and new claims 55-92 added. Support for the new claims, which are drawn to the same invention as elected Group I, is provided throughout the specification. For example, support for a transgenic animal containing a disrupted LKB1 gene such that an animal homozygous for the disrupted gene has a phenotype of embryonic lethal is supported by Example 6. Support for claims drawn to a transgenic animal that has a disrupted LKB1 gene and that develops polyposis or pigmentation on mucous membranes or skin can be found at, e.g., page 1, lines 9-11, and page 9, lines 19-23 and page 25, lines 9-21. No new matter has been added. Claims 55-92 are under examination, claims 16-54 being withdrawn from consideration as drawn to a non-elected invention.

35 U.S.C. § 112, first paragraph

Claims 1-15 have been rejected for alleged lack of enablement. These have been cancelled and new claims 55-92 are now presented. To the extent that the rejection applies to new claims 55-92, applicants respectfully traverse.

Phenotype

The Examiner asserts, "[t]he state of the art at the time of filing was such that one of skill could not predict the phenotype of transgenics" (Office Action at page 3) and recites a number of references to support the assertion. New claims 55-92 are drawn to transgenic non-human mammals that display specific phenotypes that are described in the specification. Claims 55-83 are drawn to transgenic mammals having an embryonic lethal phenotype when homozygous for a disrupted LKB1 gene. Animals having this phenotype are described in the specification, e.g., in Example 6. Claims 84-92 are drawn to transgenic mammals having a disrupted LKB1 gene and a phenotype of polyposis in the digestive tract or pigmental spot formation on mucous membranes or skin. This phenotype was known at the time of filing to be associated with a mutated LKB1 gene, i.e., in Peutz-Jeghers syndrome (PJS), and is described in the present

application (e.g., at page 1, lines 9-11, and page 9, lines 20-23). Applicants note that this phenotype has now been confirmed to occur in LKB1+/- mice: i.e., mice heterozygous for an LKB1 disruption (see, e.g., Jishage et al. 2002, Proc. Nat. Acad. Sci. USA. 99:8903-8908; Rossi et al., 2002, Proc. Nat. Acad. Sci. USA 99:12327-12332; and Bardeesy et al., 2002, Nature 419:162-167; copies attached). All of the recited phenotypes are fully supported by the specification. In view of the pending claims, all of which recite a specific phenotype, applicants believe that no prediction of phenotype is required and the claims are enabled.

Species

The Examiner also states that at the time of filing "targeted gene insertion technology was not available for any species other than mouse" (Office Action at page 5). Applicants respectfully disagree. Many examples of transgenic animals were known in the art. For example, transgenic rabbits, sheep, and pigs (e.g., Hammer et al., 1985 Nature 315:680-683, copy enclosed), and cattle (e.g., Roschlau et al. 1989, J. Reprod. Fertil. Suppl. 38:153-160, copy enclosed) had been made prior to the priority date of the present application. Such targeted gene insertion technology was well known (see, e.g., U.S. Patent No. 5,487,992, copy enclosed).

The Examiner also expresses the view that "[e]xamples in the literature aptly demonstrate that even closely related species carrying the same transgene construct can exhibit widely varying phenotypes" (Office Action at page 4). In the pending claims, a phenotype of the transgenic animal is specified. It is of no consequence to the present invention whether some animals would produce different phenotypes, only that one in the art could produce and identify an animal having the recited phenotype.

The Examiner also appears to base the rejection, in part, on the belief that embryonic stem cells are required for creating transgenic animals using homologous recombination. Applicants know of no such requirement and cannot identify such an assertion in any of the references cited by the Examiner. Indeed, the McCreath et al. reference cited in the Office Action (2000, Nature 405:1066-1069) amply demonstrates that embryonic stem cells are <u>not</u> required for production of gene-targeted sheep. McCreath et al. used cultured somatic cells as the target cells; following *in vitro* homologous recombination, nuclei from correctly targeted cells were transferred into ovine oocytes. These experiments produced three apparently healthy

transgenic lambs. Since the general techniques utilized by McCreath et al. (gene targeting by homologous recombination) in cultured somatic cells (e.g., in the context of studying the effects of knocking out a gene in the cultured cell) and nuclear transfer in oocytes (e.g., in the context of cloning farm animals) were well known and practiced at the priority date of the present application, it would appear that the evidence cited by the Examiner supports applicants' position, rather than the Examiner's. Given this evidence, as well as the presumption that every specification is adequately enabling, applicants request withdrawal of this aspect of the enablement rejection.

Induction

The Examiner states "[t]he specification fails to enable using a transgenic, non-human mammal in which the suppression of expression of an endogenous LKB1 gene is induced or can be induced" and appears to require that the transgenic animals have a phenotype that reflects a disease state (Office Action at page 6, citing the specification at page 27, lines 8-10). The Examiner goes on to state that the invention would require "undue experimentation to determine when the desired phenotype has been reached and how to use the animal model of disease." (Office Action at page 6).

As discussed above, the present claims recite a phenotype that could have been determined by one of skill in the art at the time the application was filed. Thus, no undue experimentation is required to determine the phenotype.

Regarding the use of the claimed animals, applicants point out that the cells derived from embryonic animals that have a disrupted LKB1 gene can be useful, e.g., for screening candidate pharmaceutical agents. Also useful in such methods are animals that have a phenotype such as pigmental spot formation on mucous membranes or skin or polyposis in the digestive tract as is associated with PJS, a heterozygous condition. Such uses are described in the specification at page 9, lines 24-30.

Pending claims 84-92 are drawn to animals in which LKB1 function is disrupted in a post-natal animal. The Examiner alleges that such claims are not enabled. Specifically, the Examiner states "[t]he specification does not disclose how to introduce Cre recombinase to a postnatal animal or, if a transgene were used to introduce Cre, what promoter(s) should be used

to express Cre" (Office Action at page 7). Applicants do not believe that it is necessary to provide enablement for methods of using Cre beyond those provided in the specification. For example, the specification states

> the Cre recombinase in cells can be expressed, for example, by methods employing expression vectors such as adenoviral vectors or alternatively by mating transgenic animals in which the expression of cre is regulated by a promoter capable of regulating the expression in a tissue specific or phase specific manner with mammals having the Cre-loxP system. (specification at page 8, lines 7-16).

One in the art would, at the time of filing, have been aware of methods that relate to the expression of Cre recombinase in specific tissues or postnatal animals to disrupt a selected gene. For example, Gu et al. (1994, Science 265:103-106, copy enclosed) disclosed the use of the Cre-lox system to disrupt a DNA polymerase-β gene in a particular cellular compartment (T cells).

Regulatory region

The Examiner also states that applicants "fail to enable making or using a transgenic, non-human mammal wherein the suppression of expression of an endogenous LKB1 gene is induced by deleting a regulatory region of the LKB1 gene (claims 2 and 11)" (Office Action at page 8). Applicants maintain that one of ordinary skill in the art would know that at least part of the regulatory region of any gene is upstream of the coding region. Thus, one would know to target a region upstream of the LKB1 sequence for deletion. Furthermore, one of ordinary skill in the art would know how to identify animals having such a mutation using methods described in the specification, for example by assaying for a decrease in LKB1 expression or activity. No more than routine experimentation would be required.

In view of the amended claim set and the arguments presented above, applicants request withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

35 U.S.C. § 112, second paragraph

Claims 1-15 have been rejected for alleged indefiniteness. These claims are cancelled and, as discussed below, applicants believe that the rejections do not apply to the newly presented claims.

The claims have been rejected for use of the term "can" in the phrase "suppression...can be induced." Since none of the pending claims employs this phrase, the rejection is moot.

Claim 2 has been rejected for being unclear as to whether it refers to deletion of the transgene that had been inserted into the endogenous LKB1 gene by homologous recombination or to direct deletion of the endogenous LKB1 gene. This claim has been cancelled and applicants believe that none of the pending claims retain the language referred to by the Examiner. Accordingly, the rejection is moot.

The rejection of claim 10 relates to use of the term "inducibly suppressed." This term does not appear in any of the pending claims. Applicants therefore believe that the rejection is moot.

In view of the present claim language, applicants believe that the rejections under 35 U.S.C. § 112, second paragraph are moot and request that the rejection be withdrawn.

35 U.S.C. § 102 (b)

Claim 1 has been rejected as being anticipated by Mullins (1989, EMBO J. 8:4066-4072). Claim 1 (now cancelled) and all of the currently pending claims relate to the disruption of an LKB1 gene. The Examiner states "Mullins taught a DBA/2J Ren-2 mouse in which the suppression of expression of endogenous LKB1 gene can be induced" (Office Action at page 11). Applicants thank the Examiner for her helpful comments on June 9, 2003, clarifying the rejection.

To anticipate a claim, a reference must teach every element of the claim. Applicants see no reference in Mullins to an LKB1 gene, much less, to disruption of an LKB1 gene. Clearly this reference does not disclose all limitations of any of the pending claims. Therefore, this reference cannot anticipate any of the claims. In addition, the pending claims do not use the term "can be," which the Examiner explained was the basis for the rejection.

In view of the above argument, applicants respectfully request withdrawal of the rejection under § 102 (b).

35 U.S.C. § 103 (a)

Capecchi and Hemminki

Claims 1, 4, 7, and 10-15 have been rejected for alleged obviousness over Capecchi (Sci. Amer., 1994, 270:34-41) in view of Hemminki (1998, Nature, 391:184-187). Capecchi describes a mouse whose genome contained a disruption in the HoxA-3 gene by insertion of a selective marker gene into the HoxA-3 gene. Hemminki discusses a nucleic acid sequence of an LKB1 gene. Applicants respectfully request reconsideration of the rejection.

To establish a *prima facie* case of obviousness, the prior art reference(s) must teach or suggest all the claim limitations. The Examiner stated "[n]ote that absent any phenotypic requirements for the claimed transgenic mouse, the combination of the cited prior art is sufficient to make obvious the claimed invention" (Office Action at page 12, third paragraph). All of the pending claims relate a phenotype for the claimed transgenic non-human mammal. Nothing in either Capecchi or Hemminki discloses a phenotype for a transgenic animal having a disrupted LKB1 gene. Thus, the cited art does not teach or suggest all of the claim limitations. Accordingly, applicants believe that no combination of Capecchi and Hemminki can make the claimed invention obvious.

Orban and Hemminki

Claims 1-15 have been rejected for alleged obviousness over Orban (1992, Proc. Nat. Acad. Sci. USA 89:6861-6865) in view of Hemminki (1998, Nature 391:184-187). The Examiner describes Orban as teaching a mouse whose genome comprises a β-galactosidase gene inserted between a pair of loxP sites. Orban does not mention an LKB1 gene. Hemminki describes an LKB1 sequence.

To establish a *prima facie* case of obviousness, the prior art reference(s) must teach or suggest all the claim limitations. All of the pending claims relate a phenotype for the claimed transgenic non-human mammal. Nothing in either Orban or Hemminki discloses a phenotype for

a transgenic animal having a disrupted LKB1 gene. Thus, the cited art does not teach or relate all of the claim limitations. Accordingly, applicants believe that no combination of Capecchi and Hemminki can make the claimed invention obvious.

Obviousness requires a reasonable expectation of success. The Examiner's argument that the teachings of Capecchi and Hemminki or Orban and Hemminki, none of whom made or even speculates about LKB1 knockout mammals, provide one of ordinary skill in the art with both motivation and reasonable expectation of success in making such a knockout is not consistent with the Examiner's argument related to the rejection under 35 U.S.C. § 112 for lack of enablement. The lack of enablement rejection is based, at least in part, on the Examiner's opinion that transgene behavior is unpredictable. Applicants do not see how something deemed to be unpredictable could also be considered to have a reasonable expectation of success. In view of this inconsistency, Applicants believe that it is improper to reject the claimed invention for both lack of enablement (e.g., based on the alleged lack of expectation of success) and alleged obviousness.

In view of the arguments presented above, applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 103 (a).